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Frequency of Carbapenem Resistance in the Pathogenic Gram-Negative Bacteria from Hyderabad, Sindh

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ABSTRACT

Carbapenems are β-lactam antibiotics and are often used as a last resort to treat infections caused by the β-lactamase-producing Gram-negative bacteria owing to their ability to withstand hydrolysis by many β-lactamase enzymes. However, the emergence of carbapenem resistance in these pathogens has already been reported. In order to avoid critical situations for public health, regular monitoring and reporting of carbapenem resistance is essential. **Objectives:** To determine the frequency of carbapenem-resistant Gram-negative pathogens circulating in Hyderabad, Sindh. **Methods:** This cross-sectional study was carried out for one year. The clinical samples were collected using a convenience sampling technique from patients suspected of bacterial infections. The bacterial isolates were subjected to identification based on their microscopic, cultural, and biochemical characteristics. Sensitivity of each type of Gram-negative pathogen to antibiotics was established in terms of Clinical Laboratory Standards Institute guidelines, with the use of the Kirby-Bauer disk diffusion technique. **Results:** 400 clinical samples were randomly selected and they were divided into urine (n=212), pus (n=85), blood (n=68), and other (n=35). Their microbiological processing resulted in the recovery of two hundred seventy-seven isolates of Gram-negative bacteria identified as *E. coli* (31.05%), *Enterobacter* spp. (24.19%), *Pseudomonas* spp. (16.25%), *Proteus* spp. (14.44%), *Klebsiella* spp. (10.11%), and others (3.96%). The frequency of carbapenem-resistant isolates varied among species, with the highest prevalence in *Pseudomonas* spp. demonstrating 20% being carbapenem-resistant isolates. **Conclusions:** Carbapenem resistance in pathogenic Gram-negative bacteria has emerged. The development of carbapenem resistance in these pathogens can be catastrophic for public health.

INTRODUCTION

Carbapenems, a subgroup of β-lactam antibiotics, act as cell wall inhibitors by restraining the biosynthesis of bacterial cell walls and are recognized as last-resort antibiotics for treating severe bacterial infections [1, 2]. Imipenem and meropenem are among the most broadly active carbapenem antibiotics available for systemic use in humans [3]. Carbapenems are typically resistant to hydrolysis by many beta-lactamase enzymes released by clinically significant bacterial pathogens. However, the emergence of carbapenem resistance (CR) in Gram-negative pathogenic bacteria such as *E. coli* and some species of *Acinetobacter*, *Klebsiella*, *Proteus*, and *Pseudomonas* genera has been reported in the last few years [4, 5], which has imposed a great public health concern worldwide by limiting treatment options for infections caused by CR-Gram-negative pathogenic bacteria. Consequently, infected patients face a high mortality rate. Additionally, the annual cost of combating these resistant bacterial infections has increased substantially worldwide [6, 7]. The carbapenem resistance in Gram-negative bacteria is mainly attributed to the conjugative plasmids, which can spread mobile genes that encode enzymes (carbapenemases) capable of hydrolyzing β-lactam agents, including carbapenems [8]. Among the

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clinically relevant carbapenemases, two types, namely K. *pneumoniae* carbapenemase (KPC) and Verona integron-encoded metallo-β-lactamase (VIM), are particularly prevalent. However, recently, oxacillinase-48 (OXA-48) and New Delhi metallo-β-lactamase-1 (NDM-1) have also become common [9]. Since the last decade, the isolation of CR-Gram-negative bacteria from clinical samples has been increasing, possibly due to the frequent usage of carbapenem antibiotics for treating bacterial infections. Consequently, CR-Gram-negative pathogenic bacteria have been listed in the critical priority pathogens group by the World Health Organization [10]. The epidemiological attributes of CR-Gram-negative pathogenic bacteria include multiple characteristics that show diversity and significantly vary by geographical region. Regular monitoring and surveillance of carbapenem resistance in Gram-negative pathogenic bacteria are suggested. The collected data have provided insights into the status of these clinically important antibiotics in terms of their effectiveness in treating infections associated with various Gram-negative bacteria.

Despite the growing global concern regarding carbapenem-resistant Gram-negative bacteria, region-specific surveillance data from interior Sindh, particularly Hyderabad, remain limited. Most available reports focus on national or tertiary-care urban centers, leaving a gap in localized epidemiological evidence needed to guide empirical therapy and antimicrobial stewardship strategies. The absence of updated regional resistance patterns may hinder timely clinical decision-making and infection control planning. Therefore, assessing the current frequency of carbapenem resistance in Gram-negative pathogens within this setting is essential. This study aimed to evaluate the frequency of CR in Gram-negative pathogenic bacteria from Hyderabad, Sindh, Pakistan.

METHODS

The study was conducted as a prospective and cross-sectional study in a period of one year (September 2021 to August 2022) at the Institute of Microbiology, University of Sindh, Jamshoro. The Institutional Bioethics Committee (IBC), University of Sindh, Jamshoro, Pakistan, was the source of the ethical approval through a letter with no. ORIC/SU/ 125. Cochran's sample size formula [11] was used to calculate sample size, ensuring a 95% level of confidence, a 5% margin of error and a 0.5 estimated proportion. Clinical specimens, including urine, blood, pus and pus swabs, vaginal swabs, tissue or wound aspirates, were collected from inpatients and outpatients visiting various diagnostic centers located in Hyderabad. Informed consent was obtained through verbal means directly via patients (or the guardian in the case of a minor), but no identifiable information has been shared, and the samples were gathered for diagnostic reasons. The standard convenience sampling method was used to collect the samples. The collected samples were immediately transported to the laboratory and processed on solid media (selective and differential), followed by incubating the inoculated plates at 37°C for 24 hours. The next day, the cultural characteristics were taken into account. Lactose-fermenting Gram-negative bacteria appeared pink while non-fermenters formed colorless to yellow colonies on the MacConkey agar. The isolates were further subjected to a Gram staining procedure and biochemical tests, including the Catalase test, Oxidase test, SIM test, Indole test, Citrate utilization test, Urease test, and Triple sugar iron test, to reveal their identity. Strains that did not exhibit the usual cultural and biochemical characteristics for their identification were further analyzed using commercially available API 20 E (Analytical Profile Index) strips. The API 20 E test was performed according to the manufacturer's recommendations (Biomerieux, USA). Briefly, 20 compartments on the API 20 E strip were loaded with the diluted test culture and covered with sterile oil. The strip was incubated in a moist tray overnight. The next day, the results were observed straight away for some tests, while other tests required the addition of specific reagents. The identification was determined using the API catalogue. Only one bacterial isolate was selected from each growth-positive sample, while co-infecting bacteria were excluded. AST of all isolates was performed using the Kirby-Bauer disc diffusion method [12] on Mueller-Hinton (MH) agar (Oxoid-UK) following Clinical and Laboratory Standards Institute(CLSI) guidelines [13]. Briefly, a lawn of the test cultures was prepared on the Muller-Hinton plates, followed by the deposition of commercially available antibiotic discs, including imipenem and meropenem. After incubation, the plates were observed for the inhibition zone. The size of the inhibition zone was measured, and the isolates were scored as resistant (R) or sensitive (S) to each of the antibiotics tested according to CLSI guidelines [13]. To test the quality of antibiotics, the E. coli ATCC 25922 strain was used. Statistical analysis of the given data was performed with the help of Microsoft Excel 2010 and SPSS version 26.0. The p-value was calculated by a Chi-square independence test, and a p-value of ≤ 0.05 was regarded as statistically significant.

RESULTS

The present study included 400 different types of clinical samples. The frequency of growth-positive clinical samples is given. The observed higher frequency of positive growth patterns reflects the targeted collection from suspected patients, consistent with the aim of the

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current research. Although some samples revealed mixed growth, one isolate per sample was included in the present study for identification and detection of the CR phenotype. The data has further indicated that among the growth-positive samples (n=316), a higher percentage was of urine specimens, followed by pus and blood specimens. In contrast, other specimens (ear swabs and body fluids) were less frequent. Notably, urinary tract infections (UTIs) caused by Gram-negative bacteria were significantly more prevalent than other infections (p-value<0.05). Moreover, a higher frequency of the clinical isolates (87.65%) appeared as Gram-negative bacteria with rod-shaped morphology when microscopic observations were done, while the remaining (12.35%) were Gram-positive bacteria. It was further observed that the highest frequency of Gram-negative isolates was recovered from urine samples (p-value<0.050), followed by blood and pus samples (Table 1).

Table 1: Percentage Distribution of Clinical Specimens

Clinical Samples	Gram-positive, n (%)	Gram-negative, n (%)	Total Growth Positive, n (%)
Urine	11 (28.2%)	171 (61.7%)*	182 (57.59%)*
Pus	25 (64.10%)	44 (15.9%)	69 (21.84%)
Blood	02 (5.13%)	43 (15.5%)	45 (14.24%)
Other	01 (2.57%)	19 (6.9%)	20 (6.33%)
Total	39 (100%)	277 (100%)	316 (100%)

*p-value<0.050

Further characterization of Gram-negative isolates showed that the rod-shaped bacilli were frequent among them, which included both lactose fermenters and lactose non-fermenters. The isolates identified as *E. coli*, *Enterobacter* spp., *Pseudomonas* spp., *Proteus* spp., Klebsiella spp., *Citrobacter* spp., *Salmonella* Typhi, and Xanthomonas maltophila were recovered with varying frequencies. The data indicated that the majority of isolates were responsible for causing UTIs in humans because these were obtained from urine samples. However, all *Salmonella* spp. isolated in this study were exclusively recovered from blood samples. This finding is consistent with the characterization of *S. Typhi*, which causes typhoid fever, an enteric fever primarily detected by the isolation of typhoidal *Salmonella* from human blood (Table 2).

Table 2: Percentage Distribution of the Gram-Negative Isolates

Type of Specimen	Lactose Fermenters, n=185 (66.79%)				Non-Lactose Fermenters, n=92 (33.21%)			
	E. coli	*Enterobacter* spp.	*Klebsiella* spp.	*Citrobacter* spp.	*Proteus* spp.	*Pseudomonas* spp.	*Salmonella* Typhi	*Xanthomonas maltophilia*
Pus	6 (7.0%)	12 (17.9%)	4 (14.3%)	0 (0%)	15 (37.5%)	6 (13.3%)	0 (0%)	1 (100%)
Urine	64 (74.4%)	41 (61.2%)	24 (85.7%)	4 (100%)	21 (52.5%)	17 (37.8%)	0 (0%)	0 (0%)
Blood	8 (9.3%)	9 (13.4%)	0 (0%)	0 (0%)	3 (7.5%)	17 (37.8%)	6 (100%)	0 (0%)
Other	8 (9.3%)	5 (7.5%)	0 (0%)	0 (0%)	1 (2.5%)	5 (11.1%)	0 (0%)	0 (0%)
Total	86 (100%)	67 (100%)	28 (100%)	4 (100%)	40 (100%)	45 (100%)	6 (100%)	1 (100%)
% of Total Isolates	31.05%	24.19%	10.11%	1.44%	14.44%	16.25%	2.17%	0.35%

The representative result of antibiotic susceptibility testing for Gram-negative bacteria (E. coli) using the disc diffusion assay. No zone of inhibition against the antibiotic disc (Resistant), Zone of inhibition (Sensitive). Antibiotics tested were Amikacin (AK), Cephalosporins, Cefoperazone (CEF), Cefuroxime (CXM), Ciprofloxacin (CIP), Fosfomycin (FOS), Imipenem (IMP), and Meropenem (MEM). The antibiotic resistance profile of all common clinical isolates was determined against a panel of seven antibiotics from different classes of antibiotics, including carbapenems (Imipenem and meropenem). Information indicated that the *E. coli* isolates have become resistant to antibiotics, which are mostly used. Interestingly, the majority of the *E. coli* isolates were vulnerable to carbapenem antibiotics but resistant to cephalosporins and fluoroquinolone antibiotics. A particularly high resistance rate was observed against ciprofloxacin (Figure 1).

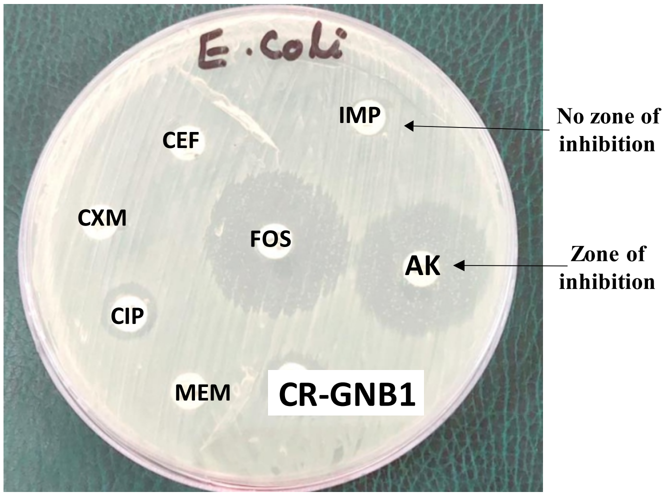

Figure 1: Antibiotic Susceptibility Testing for Gram-Negative Bacteria (*E. coli*) Using Disc Diffusion Assay

Additionally, *E. coli* isolates also showed resistance to the cephalosporin antibiotics used in the study. The CR was observed in 6.98% (n=6) of *E. coli* isolates, which is considered an emerging concern for public health. Notably,

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a higher degree of CR was observed among *Pseudomonas* spp. along with a higher level of resistance against cephalosporins such as cefuroxime and cefoperazone observed in *Pseudomonas* spp. Similarly, *Klebsiella* spp. were resistant particularly against ciprofloxacin, with 64.29% isolates. In addition, more than 50% of isolates showed resistance against both cephalosporins tested in this study. Regarding the carbapenems, 4 (14.29%) isolates showed resistance to meropenem and imipenem antibiotics. Similarly, a higher level of resistance against cephalosporins such as cefuroxime and cefoperazone was observed in Pseudomonas spp. Antibiotic susceptibility testing of *Enterobacter* spp. revealed that 46 (68.66%) were susceptible to cefuroxime, while 21 (31.34%) were resistant. Furthermore, resistance to ciprofloxacin was observed in more than 50% of isolates. Specifically, 7 (10.45%) of *Enterobacter* spp. were resistant to meropenem, a carbapenem antibiotic, indicating the emergence of CR in the Gram-negative pathogenic bacteria. AST of *Proteus* spp. isolates revealed that 31 (77.5%) were sensitive to cefuroxime and cefoperazone, while 9 (22.5%) were resistant. Additionally, resistance to ciprofloxacin was observed in 18 (45.0%) of the isolates. Furthermore, 6 (15.0%) of the *Proteus* spp. exhibited resistance to carbapenems. Overall, data showed that 32 out of 266 Gram-negative isolates tested were CR isolates.

Table 3: Antibiotic Susceptibility Profiles of Gram-Negative Bacteria Isolated from Various Clinical Samples

Clinical Isolates	***E. coli* n=86**		***Enterobacter* spp. n=67**		***Klebsiella* spp. n=28**		***Proteus* spp. n=40**		***Pseudomonas* spp. n=45**	
Antibiotics	**S, n (%)**	**R, n (%)**	**S, n (%)**	**R, n (%)**	**S, n (%)**	**R, n (%)**	**S, n (%)**	**R, n (%)**	**S, n (%)**	**R, n (%)**
Amikacin	77 (89.53%)	9 (10.47%)	58 (86.57%)	9 (13.43%)	23 (82.14%)	5 (17.86%)	35 (87.5%)	5 (12.5%)	37 (82.22%)	8 (17.78%)
Cefoperazone	56 (65.11%)	30 (34.89%)	33 (49.25%)	34 (50.74%)	14 (50%)	14 (50%)	31 (77.5%)	9 (22.5%)	23 (51.11%)	22 (48.89%)
Cefuroxime	46 (53.49%)	40 (46.51%)	46 (68.66%)	21 (31.34%)	12 (42.86%)	16 (57.14%)	31 (77.5%)	9 (22.5%)	28 (62.22%)	17 (37.78%)
Ciprofloxacin	24 (27.90%)	62 (72.10%)	31 (46.26%)	36 (53.73%)	10 (35.71%)	18 (64.29%)	22 (55.0%)	18 (45.0%)	24 (53.33%)	21 (46.67%)
Fosfomycin	75 (87.20%)	11 (12.80%)	47 (70.15%)	20 (29.85%)	18 (64.29%)	10 (35.71%)	26 (65.0%)	14 (35.0%)	25 (55.56%)	20 (44.4%)
Imipenem	80 (93.02%)	6 (6.98%)	60 (89.55%)	7 (10.45%)	24 (85.71%)	4 (14.29%)	34 (85.0%)	6 (15.0%)	36 (80.0%)	9 (20.0%)
Meropenem	80 (93.02%)	6 (6.98%)	60 (89.55%)	7 (10.45%)	85.71 (24%)	4 (14.28%)	34 (85.0%)	6 (15.0%)	36 (80.0%)	9 (20.0%)

R=Resistant and S=Sensitive.

The percentage distribution of the CR-Gram-negative isolates is shown. In the current study, a chi-square test of independence was performed to determine whether the bacterial species and carbapenem resistance were significant; the results indicated the lack of a significant association (p-value>0.05) (Figure 2).

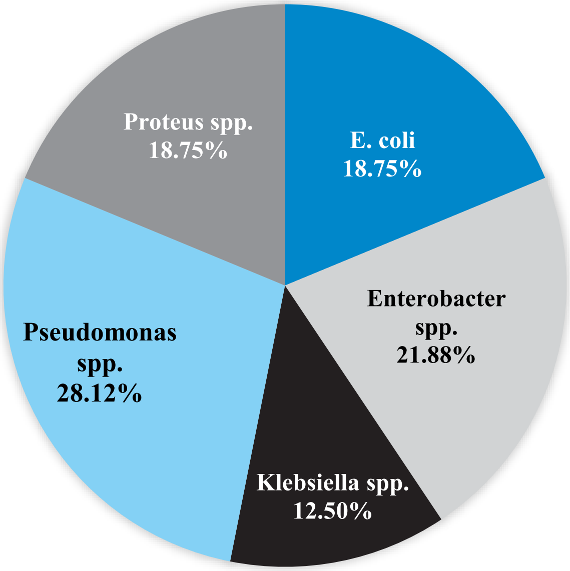

Figure 2: The Percentage Distribution of CR Gram-Negative Bacteria

DISCUSSION

Carbapenems are generally resistant to hydrolysis caused by clinically important β-lactamase enzymes, which are produced by Gram-negative bacteria. Therefore, the usage of carbapenems is commonly employed as a final resort antibiotic to treat infections that are brought about by the Gram-negative pathogens that produce β-lactamase. However, the acquisition of CR phenotype by the pathogens causes a challenging situation because of the lack of alternative treatment options for bacterial infections caused by CR Gram-negative pathogens. Consequently, CR has led to a dramatic increase in the death rate. The present study aimed to investigate carbapenem resistance developed by Gram-negative pathogens circulating in the study area. To realize the objective of the current study, firstly, Gram-negative pathogens were isolated among the patients who were suspected of bacterial infection. In our data, *E. coli* and the species of *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Proteus*, and *Salmonella* were the most ubiquitous Gram-negative pathogens that cause different bacterial infections in humans in the study area. Our data has further highlighted that UTIs were more common than other bacterial infections. These findings are in agreement with the recent study, which has indicated an increased prevalence of UTIs across a wide range of bacterial infections worldwide [14]. Furthermore, *E. coli* remains the most prevalent causative agent of bacterial infections caused by Gram-negative bacteria [15]. Similarly, lactose-fermenting Gram-negative pathogens other than *E. coli* were also found to cause UTIs. Therefore, the development of antibiotic resistance in Gram-negative pathogens will contribute to the challenging public health concern. Furthermore, CR in *Proteus* spp. and *Pseudomonas* spp. presents a more concerning situation because the resistance mechanism causing the CR phenotype in these pathogens is comparatively less described. Moreover, AST results indicated that the majority of *E. coli* isolates have acquired resistance to ciprofloxacin and cephalosporin antibiotics, which are the common treatment options for *E. coli*-associated infections in humans. These findings are supported by a previous study that reported the higher frequency of ESBL producers among Gram-negative pathogenic bacteria [16]. Furthermore, the results suggested that *E. coli* isolates in this study exhibited high susceptibility to amikacin and carbapenem antibiotics, supported by the previous studies reporting minimal resistance against these antibiotics among clinical *E. coli* isolates [17]. However, the highest frequency of CR was observed in *Pseudomonas* spp. Overall, data show that CR is emerging in Gram-negative pathogens, which means that our last resort antibiotics are gradually failing to provide treatment for bacterial infections, thus limiting the antibiotic choices to treat infections associated with MDR-Gram-negative pathogens. Our data is consistent with recent reports from Pakistan showing an increasing trend of MDR in Gram-negative pathogens [18, 19]. Better monitoring and increased detectability of CR-Gram-negative pathogens underscore the fact that infections attributed to said pathogens are associated with serious morbidity and death [20]. In this regard, the current work explains the significance of researching the mechanisms of CR among Gram-negative pathogens found in the region of the current study. This could potentially facilitate the undetected spread of antibiotic-resistant pathogenic strains within our hospitals. Consequently, public health might experience a challenging situation sooner or later. Therefore, routine detection of these isolates should be prioritized. To achieve this, laboratories must possess the capacity to quickly identify these isolates, enabling the implementation of appropriate therapy to prevent antibiotic misuse or overuse. Furthermore, it is advisable to implement effective control measures to facilitate proper management and reduce the spread of these organisms.

This study was limited by its single-center design and reliance on phenotypic susceptibility testing without molecular characterization of carbapenemase genes. Additionally, the use of convenience sampling may restrict the generalizability of the findings to the wider population. Future studies should incorporate multicenter surveillance, molecular detection of resistance genes (such as blaKPC, blaNDM, and blaOXA-48), and long-term trend analysis to better understand transmission dynamics and support targeted antimicrobial stewardship interventions.

CONCLUSIONS

It was concluded that resistance against last-resort antibiotics like carbapenems has emerged in Gram-negative pathogens. Consequently, it is crucial to exercise prudent use of carbapenems and administer them appropriately.

Authors' Contribution

Conceptualization: MAI, SB, SAT
Methodology: MAI, SB, SAT, HD
Formal analysis: MAI, AAM, HD
Writing and Drafting: MAI
Review and Editing: MAI, AAM, HD, SB, SAT

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

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REFERENCES

[1] Hawkey PM and Livermore DM. Carbapenem Antibiotics for Serious Infections. British Medical Journal. 2012 May; 344. doi: 10.1136/bmj.e3236.

[2] Coulthurst SJ, Barnard AM, Salmond GP. Regulation and Biosynthesis of Carbapenem Antibiotics in Bacteria. Nature Reviews Microbiology. 2005 Apr; 3(4): 295-306. doi: 10.1038/nrmicro1128.

[3] Armstrong T, Fenn SJ, Hardie KR. JMM Profile: Carbapenems: A Broad-Spectrum Antibiotic. Journal of Medical Microbiology. 2021 Dec; 70(12): 001462. doi: 10.1099/jmm.0.001462.

[4] Codjoe FS and Donkor ES. Carbapenem Resistance: A Review. Medical Sciences. 2017 Dec; 6(1): 1. doi: 10.3390/medsci6010001.

[5] Nordmann P and Poirel L. Epidemiology and Diagnostics of Carbapenem Resistance in Gram-Negative Bacteria. Clinical Infectious Diseases. 2019 Nov; 69(Supplement_7): S521-8. doi: 10.1093/cid/ciz824.

[6] Dadgostar P. Antimicrobial Resistance: Implications and Costs. Infection and Drug Resistance. 2019 Dec: 3903-10. doi: 10.2147/IDR.S234610.

[7] Tillotson GS and Zinner SH. Burden of Antimicrobial Resistance in an Era of Decreasing Susceptibility.

Expert Review of Anti-Infective Therapy. 2017 Jul; 15(7): 663-76. doi: 10.1080/14787210.2017.1337508.

[8] Hussain HI, Aqib AI, Seleem MN, Shabbir MA, Hao H, Iqbal Z *et al.* Genetic Basis of Molecular Mechanisms in Β-Lactam Resistant Gram-Negative Bacteria. Microbial Pathogenesis. 2021 Sep; 158: 105040. doi: 10.1016/j.micpath.2021.105040.

[9] Patel G and Bonomo RA. Stormy Waters Ahead: Global Emergence of Carbapenemases. Frontiers in Microbiology. 2013 Mar; 4:48. doi: 10.3389/fmicb.2013.00048.

[10] World Health Organization. Prioritization of Pathogens to Guide Discovery, Research and Development of New Antibiotics for Drug-Resistant Bacterial Infections, Including Tuberculosis. 2017.

[11] Nanjundeswaraswamy TS and Divakar S. Determination of Sample Size and Sampling Methods in Applied Research. Proceedings on Engineering Sciences. 2021 Mar; 3(1): 25-32. doi: 10.3389/fmicb.2013.00048.

[12] Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic Susceptibility Testing by A Standardized Single Disk Method. American Journal of Clinical Pathology. 1966 Apr; 45(4_ts): 493-6. doi: 10.1093/ajcp/45.4_ts.493.

[13] Clinical and Laboratory Standard Institute (CLSI). Performance Standards for Anti-Microbial Susceptibility Testing. 30th Edition, M100. 2020.

[14] Öztürk R and Murt A. Epidemiology of Urological Infections: A Global Burden. World Journal of Urology. 2020 Nov; 38(11): 2669-79. doi: 10.1007/s00345-019-03071-4.

[15] Urooj S, Bano S, Tunio SA, Memon BA, Abbasi SM, Rajput Z. High Frequency of Gram-negative Bacilli (GNB) Pathogens in Wounds and Other Clinical Specimens: A Grave Public Health Concern. BioScientific Review. 2024 May; 6(2): 20-30. doi: 10.32350/bsr.62.2.

[16] Abassi SM, Bano S, Tunio SA, Memon BA, Korejo IA. Frequency of Extended Spectrum Β Lactamases Producers in Gram-Negative Bacilli Pathogens. Rawal Medical Journal. 2024 Apr; 49(2): 255-.

[17] Niranjan V and Malini A. Antimicrobial Resistance Pattern in Escherichia Coli Causing Urinary Tract Infection among Inpatients. Indian Journal of Medical Research. 2014 Jun; 139(6): 945-8.

[18] Abro UA, Bano S, Tunio SA, Abassi SM. Antibiotic Resistance Trends in High-Risk Lineages of Salmonella Enterica Serovar Typhi: A Study Spanning Pre to Post COVID-19 Pandemic. Infection, Genetics and Evolution. 2024 Sep; 123: 105632. doi: 10.1016/j.meegid.2024.105632.

[19] Detho H, Bano S, Tunio SA, Abbasi SM, Ahmed M. Biofilm Formation and Antibiotic Resistance in Uropathogenic Escherichia Coli: The Quest for Effective Treatment of Urinary Tract Infections. Pure and Applied Biology. 2023 Nov; 13(1): 38-46. doi: 10.19045/bspab.2024.130004.

[20] Hauck C, Cober E, Richter SS, Perez F, Salata RA, Kalayjian RC *et al.* Spectrum of Excess Mortality Due to Carbapenem-Resistant Klebsiella Pneumoniae Infections. Clinical Microbiology and Infection. 2016 Jun; 22(6): 513-9. doi: 10.1016/j.cmi.2016.01.023.